

PHYTOCHEMICAL AND FTIR SPECTROSCOPIC ANALYSIS OF *Balanites aegyptiaca* SEED AND STEM BARK EXTRACT

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ABSTRACT

With various applications, *Balanites aegyptiaca* (Desert Date) is a traditional remedy for jaundice, intestinal worm infection, syphilis, malaria, dysentery amongst others but despite its good medicinal properties, it is still considered a lost crop because little or no attention has been put into using it for generation of modern medicines. Hexane and Methanol Extracts from seed and Stem Bark of the plant were analyzed for the presence of phytochemicals and the extract and isolates were also analyzed using Fourier Transform Infrared Spectrophotometer. The result of the analysis revealed the presence of secondary metabolites such as alkaloids, flavonoids, saponins, steroids, resin and other bioactive compounds. The seed FT-IR analysis of *Balanites aegyptiaca* showed peaks at 3240.63 cm⁻¹ which corresponded to an O-H stretching typical for an aromatic ring. The peaks at 3567.36 cm⁻¹, 2921.98 cm⁻¹, 2359.42 cm⁻¹, 1743.48 cm⁻¹, 1653.33 cm⁻¹ and 1130.91 cm⁻¹ corresponded to C≡C stretching typical for alkyne, C-H stretching typical for alkane, C-O stretching typical for Ester, C-C stretching typical for alkane and C-O bending typical for carboxylic acid. The stem bark FT-IR analysis of *Balanites aegyptiaca* showed peaks at 3396.29 cm⁻¹ and 3374.85 cm⁻¹ representing the bonded -OH group corresponding to aromatic rings of phenol. Peaks at 1065.00cm⁻¹, 1734.06cm⁻¹, 1706.71cm⁻¹, 1230.95cm⁻¹, 1175.12cm⁻¹ and 1734.06cm⁻¹ was associated to C-O stretching typical for esters. The study would help in developing new economic opportunities for rural communities through cultivation, processing, and commercialization of the plant. Additionally, it could promote environmental sustainability by encouraging the use of locally available resources.

Keywords: *Balanites aegyptiaca*, hexane extract, methanol extract, phytochemical analysis, FT-IR spectroscopy.

INTRODUCTION

Diabetes is a major global public health problem that affects both industrialized and developing countries. A modern, sedentary lifestyle brought on by modernization and Westernization, increased personal obesity, and certain genetic predispositions are the main causes of the rise in diabetes [1]. For type I diabetes mellitus (DT1), insulin replacement therapy is the primary treatment; for type II diabetic

mellitus (DT2), blood glucose levels can be lowered with the help of oral hyperglycemic drugs, dietary adjustments, and lifestyle changes [2]. Globally, DT2 affects around 462 million people, making up more than 90% of all diabetics. It is estimated that 193 million people have diabetes that has not been diagnosed. It is predicted that by 2045, there will be an additional 700 million [3]. According to WHO

[4], the number of individuals with diabetes is forecast to rise from 46 million to 119 million in South-East Asia, while the prevalence of the disease is estimated to increase from 31 million to 79 million in India.

B. aegyptiaca is an auspicious therapeutic plant. The usage of many *B. aegyptiaca* parts in medicine today, including leaves, barks, roots, fruits, and kernels, indicates its medicinal use. Because of its distinct chemical compositions, *B. aegyptiaca* is widely used in traditional medicine. According to Zaahkouk et al [5] *B. aegyptiaca* extracts demonstrated anti-diabetic properties, insecticidal potential, antimicrobial activity against numerous bacterial and fungal pathogen strains, antiviral and antiparasitic activity, anti-plasmodial activity, hepatoprotective effect, and antioxidant potentiality [6].

The desert date, or *Balaniteaegyptiaca* (Linn.), is a genus of flowering plants in the caltrop family. The majority of African nations are home to the significant multipurpose tree *Balanites aegyptiaca* [7]. It is a 10-meter-tall woody evergreen xylophytic tree that grows in a variety of ecological settings. It is mostly found in semi-arid regions of tropical Africa, particularly in Senegal and Sudan, as well as Asian nations like India [8]. It is referred to as lalob, hidjihi, inteishit, and heglig in Arabic. It is known as Aduwa in Hausa and Mchunju and Bedena in Swahili and Amharic, respectively [9]. From previous literature, high concentrations of saponins, alkaloids, flavonoids, tannins, fixed oils, and phenolics were found in various portions of *B. aegyptiaca* [10- 14].

Cinnamic acid, benzoic acid, p-coumaric acid, gentisic acid, vanillic acid, ferulic acid, syringic acid, and sinapic acid are among the several nonflavonoid polyphenols that were separated from *B. aegyptiaca*. 3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-1-propanone, 2-methoxy-4-vinylphenol, 2-methoxy-3-(2-propenyl)-phenol, 2,6-dimethoxyphenol, and 2-methoxy-4-(1-propenyl)-phenol-phenol, caffeic acids, 2,4-ditert-butyl-phenol, 2,6-ditert-butyl-phenol, etc have a number of therapeutic qualities, including antibacterial, antioxidant, and anti-cancer effects, as well as antidiabetic and other protective effects [15-18]. Most notably, the fruit pulp is used in Egypt and Sudan as an antidiabetic medication and to treat helminthes infections, while the seed extract is used to treat bilharzia. Several ethnopharmacological investigations have also demonstrated that all portions of *B. aegyptiaca* have therapeutic qualities. Abdominal discomfort and asthma are treated with the root. As an antijundice medication, the plant's bark is used to treat syphilis and malaria [19- 22]. With up to 4% of sperm, an aqueous fruit extract showed spermicidal effectiveness in humans without causing local vaginal pain.

Long-term use of the fruit pulp extract caused dogs' testicles to become impaired due to hyperglycemia. Seed has antibacterial, antifungal, and expectorant properties. Leukoderma, whooping cough, and other skin conditions can all be treated with fruit. The seed has a febrifuge function. Extracts from roots have a minor protective effect against experimental malaria. An infusion of roots is used as an emetic in Kenya. 10g of seed powder is taken with a glass of water in the case of asthma [23- 25]. The research aims to

evaluate the phytochemical and FTIR Spectroscopic analysis of *Balanites aegyptiaca* Seed and stem bark extract. This would help in developing new economic opportunities for rural communities

through cultivation, processing, and commercialization of the plant. Additionally, it could promote environmental sustainability by encouraging the use of locally available resources.



Figure 1. Picture showing the (a) Seed of *B. aegyptiaca*. (b) it's Stem Bark

MATERIALS AND METHODS

Sample Collection and Identification

The seed and Stem Bark of *Balanites aegyptiaca* were obtained from Purdang, Demsa Local Government Area of Adamawa state, North Eastern Nigeria. It was then transferred into a polythene bag and transported to the Department of Plant Science and Biotechnology Nasarawa State University Keffi, Nasarawa State where it was identified by an expert.

Sample Preparation

The seed of *Balanites aegyptiaca* was crushed using a hammer in order to get the seed inside the fruit. The collected seed and Stem Bark were washed using clean water several times to remove soil, dust or dirt and dried under shade

for about 14 days. Exposure to sunlight was avoided to prevent the loss of active components. The dried samples were then pulverized with mortar and pestle and sieved into powder before storing in airtight plastic containers.

Extraction

The hexane extract was obtained by macerating 84grams of the pulverized seed sample and 82 grams of the stem bark sample in hexane for 72 hours while shaking it occasionally. The mixtures were then filtered using Whatman filter paper and the filtrate were collected and stored in separate air tight container and they were all labelled accordingly for further analysis. The same procedure was repeated using methanol as a solvent.

Phytochemical Analysis

The methods employed by Harborne [26] were used for the analysis with slight modifications by Tripathi IP and Mishra [27] and Alhassan *et al.*, [28].

Alkaloids (Wagner's Test)

Each of the various extracts (2mL) was measured into separate test tubes and 5 drops of wagner's reagent was then added to each test tube. The formation of orange colour indicates the presence of alkaloids.

Flavonoids (Lead Acetate Test)

About 2ml each of the extracts were measured into separate test tubes and 2mL of magnesium sulphate was added and 4 drops of hydrochloric acid was also added. Formation of cream light yellow colour indicated the presence of flavonoids.

Tannins (Ferric Chloride Test)

10% Ferric chloride (FeCl_3) was added in drops to 2ml each of the various Stem Bark and seed extracts in separate test tubes using a dropper. A deep bluish or greenish Colour indicated the presence of tannins.

Saponins (Foam Test)

Each extracts (1mL) was combined with 4mL of distilled water and shaken vigorously. Formation of honey comb froth indicated the presence of saponins.

Terpens and Steroids (Burchard's Test)

To 1ml each of extracts in separate test tubes, 1ml of concentrated Tetraoxosulphate (VI) acid (H_2SO_4) was added along the side of the test tubes. Formation of reddish-brown ring colour at the interphase indicated the presence of terpens and steroids.

Balsam (General Test)

Each extracts were measured in 2 mL and placed in separate test tubes. 3 drops of Alcoholic Ferric Chloride was added and observed for the formation of dark green colour which indicated the presence of balsam.

Carbohydrates (Benedict's Test)

Each extract were measured in 2mL and placed in separate test tubes and 5 drops of Benedict's reagent was also added. The mixture was then placed on a hot plate for 5 minutes and observed for the formation of brick red precipitate which indicated the presence of carbohydrates.

Phenols: 2ml each of extracts were measured into a test tube and 2ml of Ferric Chloride (FeCl_3) was also added. A change in the mixture to deep bluish green colouration indicated the presence of phenols.

Resins: 2ml of Acetic Anhydride and 4 drops of H_2SO_4 were added to each extract in separate test tubes. A formation of violet colouration indicated the presence of resins.

Thin Layer Chromatography (TLC)

TLC analysis was carried out using TLC plates coated with silica. A solvent system was prepared using different ratios of a mixture of ethyl acetate and ethanol. Spots were then made

on the plate from the raw extracts on the TLC plate and the plate was developed in the solvent system. When it got to the solvent front, the plate was removed from the solvent system and a non-polar mobile phase of Iodine crystal tank was used for detection of spots on the plate.

Column Chromatography (CC)

For column chromatography, a glass column was used and the sorbent used was silica gel. The Silica gel slurry was prepared by dissolving silica gel (60g) with methanol. The column was prepared by first putting a stationary phase of sand and then a slurry mixture of silica gel and solvent was then poured into the glass column. It was then allowed to settle down and excess solvent was removed. 20mL of the extract was introduced onto the top of the packed column and a little quantity of the stationary phase was then added. The column was eluted with a solvent system made of 60ml ethanol and 40mL ethyl acetate which was the most suitable from

the TLC plate earlier analyzed. The column's valve was then opened and about 10ml fraction of the solvent coming out from the column was collected into clean containers. Eluents which were gotten from the column fractions were examined using TLC plates in the solvent systems to obtain the retention factor (R_f) of any components that appeared as spots. The combined fractions which contained the same single component were then allowed to air-dry or evaporate to dryness to obtain a pure secondary metabolite.

FT-IR spectroscopic Analysis

Raw extracts of methanol and hexane Seed and Stem Bark samples were transferred into an air tight container and transported to Shetsco in Abuja for FT-IR spectroscopic analysis. The spectrum was then interpreted and the functional groups present in the spectrum were recorded and tabulated.

RESULTS AND DISCUSSION

Table 1: Phytochemical Analysis of *Balanites aegyptiaca* Seed and Stem Bark

Phytochemicals	Methanol Extract		Hexane Extract	
	Stem Bark	Seed	Stem Bark	Seed
Alkaloids	+	+	+	+
Flavonoids	+	-	+	+
Tannins	+	+	+	+
Saponins	-	+	-	+
Terpenes & Steroids	+	+	+	+
Balsam	+	-	+	-
Carbohydrate	+	+	+	+
Phenol	+	+	+	+

Resin	+	-	+
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Key: + (present) – (absent)

The result for the phytochemical screening of the methanolic extracts of Desert date seed as shown in table 1 above revealed the presence of alkaloids, flavonoids, saponins, tannins, carbohydrate and phenol while for the methano stem bark extract, alkaloids, tannins, phenol and resin were present. For the hexane extract of the seed, it revealed the presence of alkaloids, flavonoids, saponins, terpene & steroids and carbohydrate while the stem bark revealed the presence of alkaloids, tannins, terpenes and steroids, phenol and resin. The presence of these phytochemicals reveals that the parts of the plant analyzed have good therapeutic values and as such, *Balanites aegyptiaca* is of medicinal importance. The presence of alkaloids indicates that it can be used in generation of medicine since it is non-toxic and has important pharmacological activity such as reduction of blood pressure, killing of tumour cells, analgesic activity, circulation and respiration [29]. Saponins are non-poisonous to humans but at high percentage or dosage they are believed to cause gastroenteritis. They have many health benefits and thus can be used in production of medicinal soaps [30]. Flavonoids serve as

flavouring ingredients of spices and vegetables but their presence also means that the plant analyzed has anti-oxidant, anti-inflammatory, anti-microbial, anti-viral, anti-allergic, anti-cancer and anti-diarrheal activities [31]. Steroids possess many interesting medicinal, pharmaceutical and agrochemical activities ranging from antibacterial, anti-tumour, antihelminthic, immunosuppressive, sex hormonal and cardiogenic activity [32]. This therefore reveals that *Balanites aegyptiaca* is a plant of highly medicinal importance. In a recent study, eleven morphologically different fruits of *Balanites aegyptiaca* were analyzed by spectrophotometer combined with multivariate data analysis for secondary metabolites, the result of the analysis showed higher content of carbohydrates and lower content of other detected metabolites for group 1, moderate content of detected metabolites for group 2 and higher content of all detected metabolites for group 3. It was reported that *Balanites aegyptiaca* different parts have many bioactive metabolites such as saponins, terpenoids, alkaloids, flavonoids and steroids which have significant roles in the potentiality of *Balanites*

aegyptiaca as traditional medicine, as antimicrobial, antidiabetic, antiviral, antihelminthic, anticancer agent.

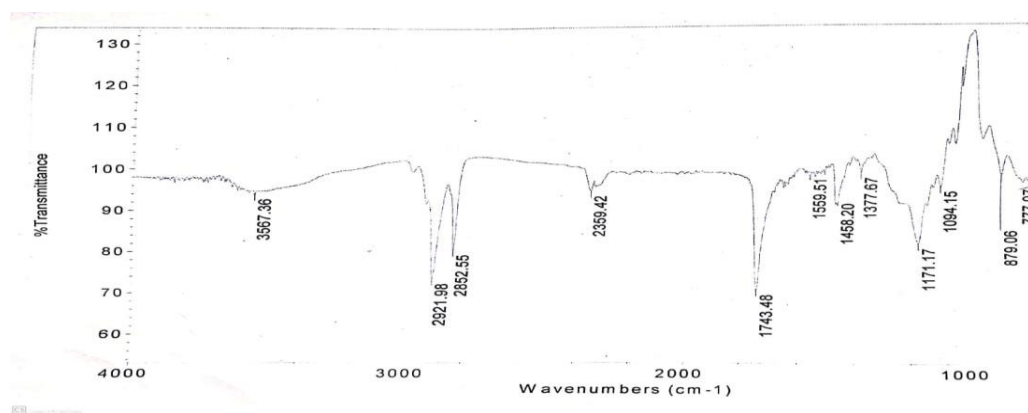


Fig 1: FTIR Spectrum of Balanites *aegyptiaca* Seed hexane extract

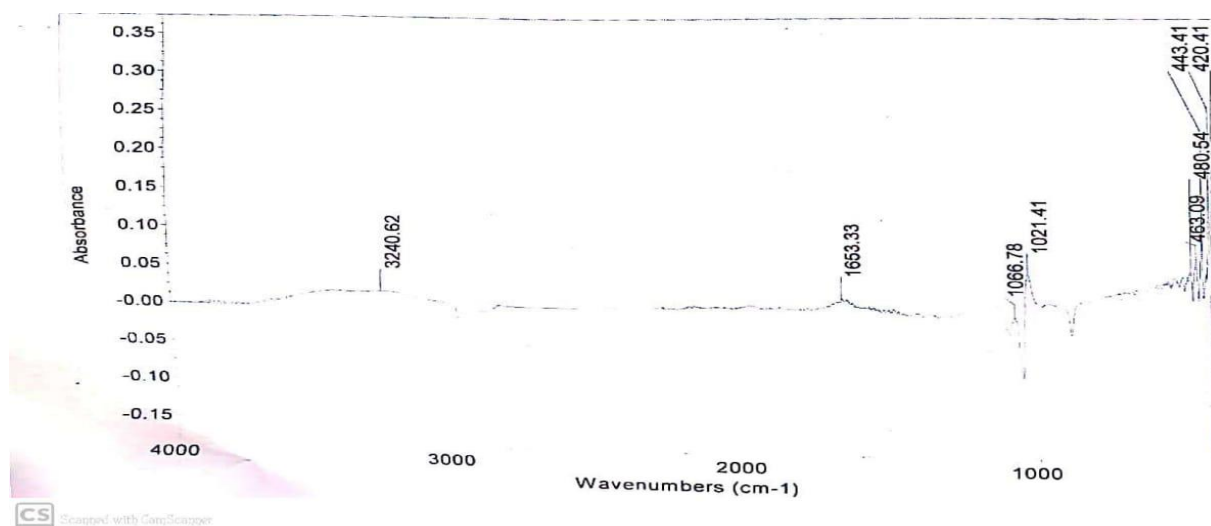


Fig 2: FTIR Spectrum of Balanites *aegyptiaca* Seed Methanol extract

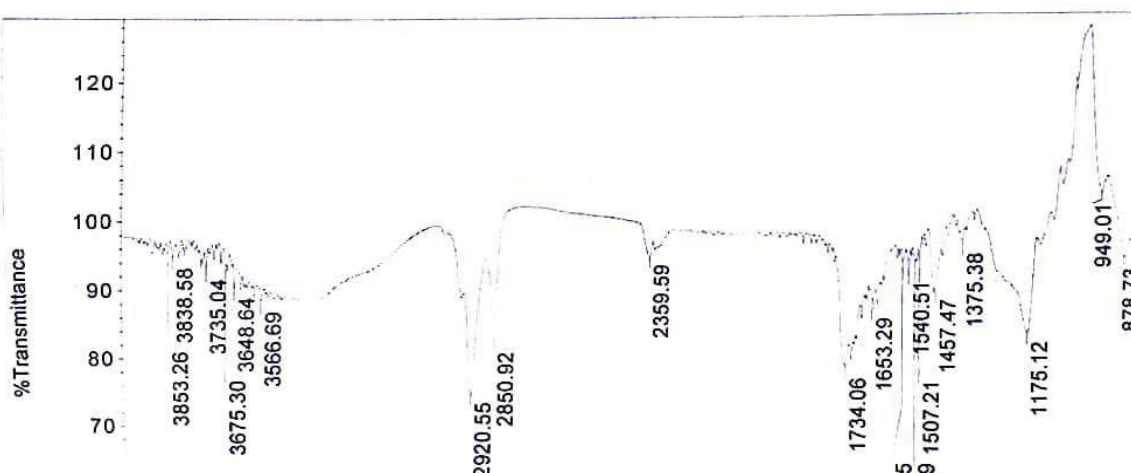


Fig 3: FTIR Spectrum of *Balanites aegyptiaca* Stem Bark hexane extract

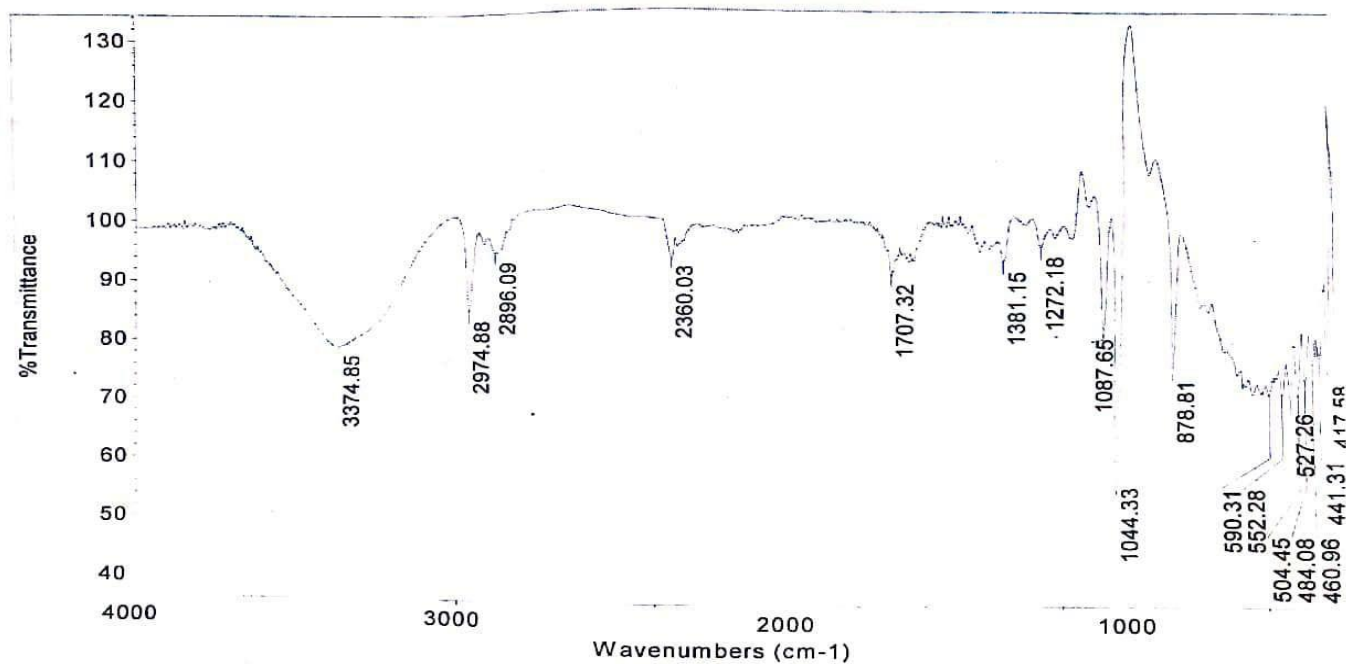


Fig 4: FTIR Spectrum of *Balanites aegyptiaca* Stem Bark Methanol extract (Isolate A)

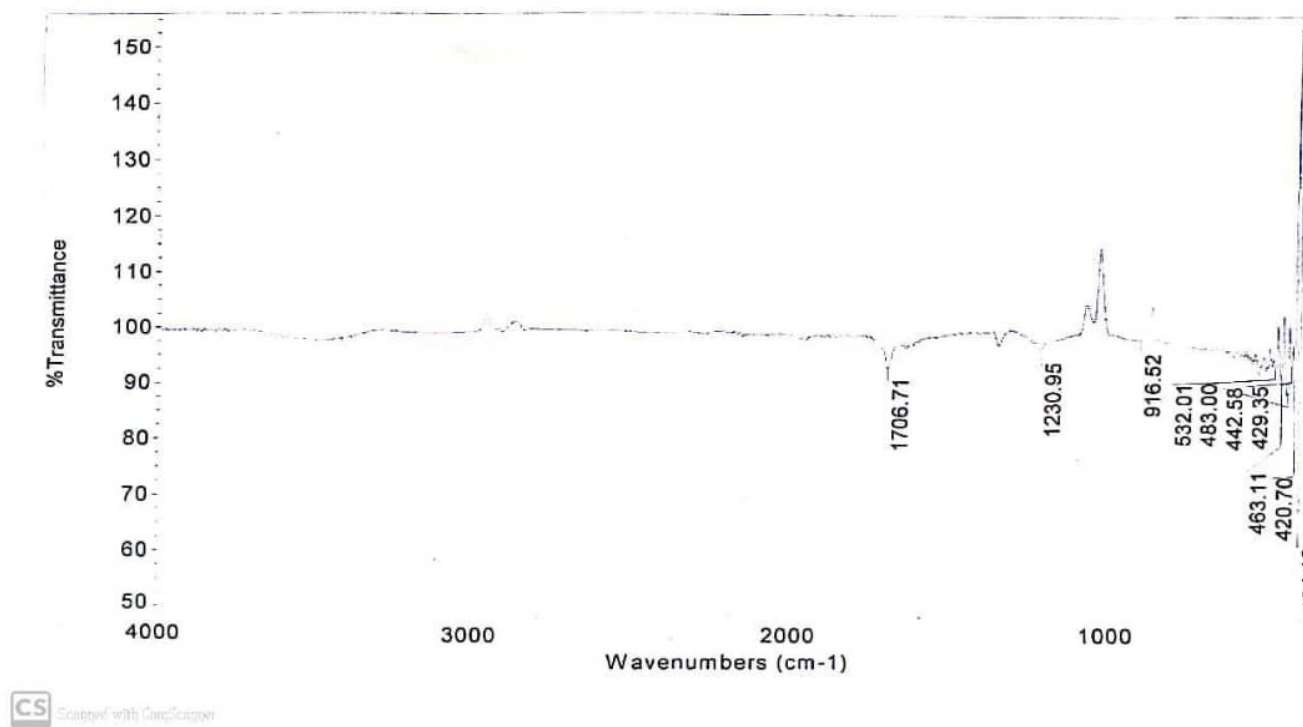


Fig 5: FTIR Spectrum of *Balanites aegyptiaca* Stem Bark Methanol extract (Isolate B)

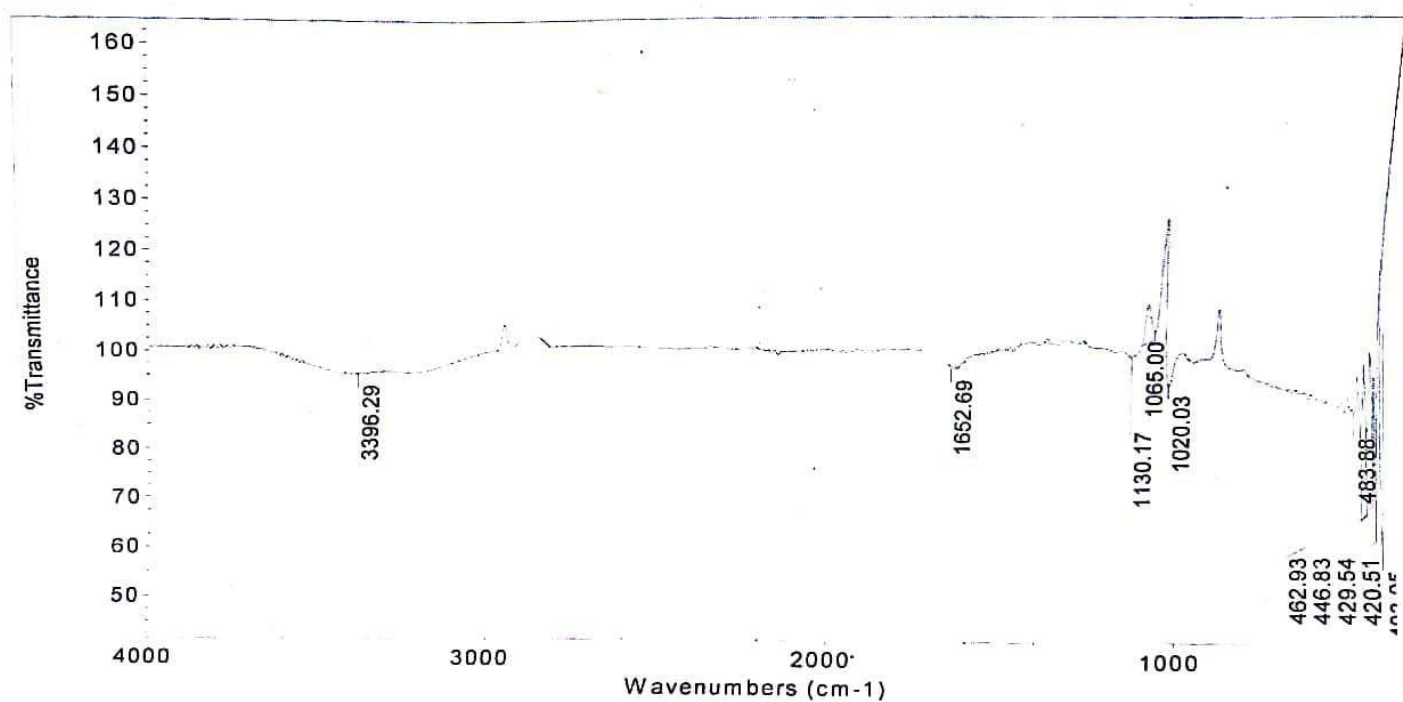


Fig 6: FTIR Spectrum of *Balanites aegyptiaca* Stem Bark Methanol Raw extract**Table 2:** Result for infrared spectrum of Desert Date Seed Raw Extract and Isolate

Sample	Vibrational Frequency (cm ⁻¹)	Assignment of bands	Functional group
Methanol	3240.62	C-O stretch, N-H stretch	Amide, amine
Extract	1653.33	C-C stretch	Alkane
	1066.78	C-N bend	Amide
	1021.41	C-O bend	Amide
Hexane Raw	3567.36	C≡C stretch	alkyne
Extract	2921.98	C-H stretch	alkane
	2852.55	C-H stretch	alkane
	1743.48	C-O stretch	Amide
	1171.11	C-O stretch	Amide
	2359.42	C≡C bend	alkyne
	1559.51	C-N bend	Amine
	777.07	C-H bend	Aromatic ring
	879.06	C-H bend	Aromatic ring

The FT-IR spectra of the extracts (methanol and hexane) of *Balanites aegyptiaca* seed as observed both from the functional and fingerprint region as shown in table 2 showed peaks at 3240.63 cm⁻¹ which corresponded to an O-H stretching typical for an aromatic ring. The peaks at 3567.36 cm⁻¹, 2921.98 cm⁻¹, 2359.42 cm⁻¹, 1743.48 cm⁻¹, 1653.33 cm⁻¹ and 1130.91 cm⁻¹ corresponded to C≡C stretching typical for alkyne, C-H stretching typical for alkane, C-O stretching typical for Ester, C-C stretching typical for alkane and C-O bending typical for

carboxylic acid. Peaks at 1066.78 cm⁻¹ and 1559.51 cm⁻¹ was also assigned to C-N bending typical for Amides. The result suggests that *Balanites aegyptiaca* extracts includes a variety of physiological active functional groups including Amine, amides, alcohol/phenols etc. (Table 2). As a result, we can prove that the plant contains significant bioactive phytochemicals. The stem bark FT-IR analysis of *Balanites aegyptiaca* as shown in table 5 showed peaks at 3396.29 cm⁻¹ and 3374.85 cm⁻¹ representing the bonded –OH group corresponding to aromatic

rings of phenol. Peaks at 1065.00cm^{-1} , 1734.06cm^{-1} , 1706.71cm^{-1} , 1230.95cm^{-1} , 1175.12cm^{-1} and 1734.06cm^{-1} was associated to C-O stretching typical for esters. Vibrational frequencies of 2974.88cm^{-1} , 2896.09cm^{-1} , 916.52cm^{-1} , 2920.92cm^{-1} and 720.22cm^{-1} corresponding to C-H stretching and bending typical for alkane and alkene were also shown. Other functional groups included $\text{C}\equiv\text{N}$

stretching typical for nitriles at vibrational frequencies of 2360.03cm^{-1} and 2359.59cm^{-1} . The result suggests that *Balanites aegyptiaca* extracts includes a variety of physiological active functional groups including Amine, amides, alcohol/phenols etc. (Table 3). As a result, we can prove that the plant contains significant bioactive phytochemicals.

Table 3: Result for infrared spectrum of Desert Date Stem Bark Raw Extract and Isolate

Sample	Vibrational Frequency (cm^{-1})	Assignment of bands	Functional group
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Methanol Raw	3396.29	N-H stretch, -OH bonded	Amine, phenol
Extract	1652.69	C-C stretch	Alkane
	1130.17	C-N bend	Amine
	1065.00	C-O bend, C-N bend	Amide, Amine
	1020.03	C-N bend	Amine
Methanol	3374.85	-OH bonded	Phenol
Extract Isolate A	2974.88	C-H stretch	Alkane
	2896.09	C-H stretch	Alkane
	2360.03	C≡N stretch	Nitriles
	1707.32	C-O stretch	Amide
	1044.33	C-N stretch	Amine
Methanol	1706.71	C-O stretch	Amide
Extract Isolate B	1230.95	C-O stretch	Amide
	916.52	C-H stretch	Alkene
Hexane Raw Extract	2359.59	C≡N stretch	Nitriles
	2850.92	C-H stretch	Alkane
	2920.55	C-H stretch	Alkane
	1175.12	C-O stretch	Amide
	1734.06	C-O stretch	Amide
	720.22	C-H bend	Alkane

CONCLUSION

From the study, it is evident that *Balanites aegyptiaca* contains bioactive compounds which shows its good usage in medicine. The suitable solvent system of 40% ethyl acetate and 60% ethanol is best for developing the TLC plate and isolation using the solvent system ratio. The functional groups present in the plant parts (Seed and Stem Bark) of *Balanites aegyptiaca* includes C≡C, C-N, C=O, C-O and –OH aromatic groups which were all observed at different peaks. Thus, the seed and stem bark of *Balanitesaegyptiaca* are

plant parts suitable for generation of drugs which will serve as supplements as well as aid in treating ailments.

Balanitesaegyptiaca (Desert Date) revealed the presence of bioactive compounds and secondary metabolites as well as functional group which are of interest in medicinal science; it is therefore recommended that more research should be carried out on the proximate composition of the plant so as to ascertain the nutritional value of the plant. Also, antibacterial and antifungal studies are recommended in order to ascertain the

pharmacological activities of the plant as well as how best it can be used by research institutes and pharmaceutical industries.

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